Early effects in kidney enzyme activities after irradiation.

FROM THE DEPARTMENT OF RADIOLOGY, SCHOOL OF MEDICINE, UNIVERSITY OF FLORENCE, I-50134 FLORENCE, ITALY.

EARLY EFFECTS IN KIDNEY ENZYME ACTIVITIES AFTER IRRADIATION

F. FRANCIOLINI, A. BECCIOLINI, A. ROSSI, V. GIACHÈ, M. BALZI and A. NARDINO

Irradiation of the kidney with sublethal doses does not reveal any acute irradiation injury of morphologic structures because of the low proliferative activity and turnover of the renal parenchyma.

Only a few reports on biochemical modifications have appeared. Disaccharase activities have been revealed in the brush border of the proximal tubules (SACKTOR 1968, SILVERMANN 1973, SILVERMANN & BLACK 1975) and in a preliminary report some results of irradiation have been published (BECCIOLINI et coll. 1976 a).

The present report concerns biochemical modifications occurring during the first five days following irradiation. In addition, some lysosomal enzyme determinations were made as well as histologic examination in order to assess cell involvement.

A dose high enough to induce a sublethal gastrointestinal syndrome was used for the experiments.

Material and Methods

Female Wistar rats 10 to 12 weeks old and weighing 160 to 180 g were used. The animals were kept on standard laboratory diet and light-darkness (L/D) cycle 6.30 a.m. to 6.30 p.m.

The rats were whole-body irradiated with a dose of 8 Gy (800 rad) using a telecobalt therapy unit.

Submitted for publication 26 July 1978.

First experiment. Forty rats were irradiated between 3.30 and 4.30 p.m. and killed after 2½, 3, 4, 6, 10, 14, 20, 26, 30, 38, 50, 74 h. Forty-three rats, used as controls, were sham-irradiated and killed at the same intervals.

Second experiment. The animals were killed at two fixed intervals after being irradiated at increasing intervals. Fifty-six rats were used, 10 of them as controls, divided into 2 groups and killed at 10 p.m. and 2 a.m., respectively. The group killed at 10 p.m. was irradiated 4, 8, 16, 24, 36, 48, 72 and 124 h before killing. The other group, killed at 2 a.m., was irradiated 8, 12, 20, 28, 40, 52, 76 and 128 h before killing.

Immediately after death the kidneys were removed. The upper part of the right kidney was fixed in Carnoy and used for morphologic observations. The sections were stained with PAS-hematoxylin. The left kidneys were weighed and homogenized with distilled water at 5% w/v. After centrifugation at 900 × g the supernatant was assayed to determine the activities of maltase (DAHLQUIST 1964), leucinaminopeptidase (LAP) (NAGEL et coll. 1964), alkaline phosphatase (BESSEY et coll. 1946), β-glucuronidase (DESAI 1969) and cathepsin D (DESAI). Other disaccharases such as trehalase and invertase appeared absent in rat kidneys. Protein content was also determined (EGGSTEIN & KREUTZ 1955). The same methods previously used for intestinal enzyme assays appeared valid also for these activities of the kidneys after slight modifications. Each sample was assayed twice: the difference between the two determinations was less than 5 per cent.

Enzyme activities were expressed in unit per g of protein, where one unit converts 1 μmol of substrate per min at 37°C. Protein content was expressed in mg/g of tissue (wet weight).

Statistical significance between the controls and the irradiated rats was determined by means of Student's t-test.

Results

First experiment. Results of maltase, alkaline phosphatase and LAP activities are given in Fig. 1. No significant circadian fluctuations appeared in the controls. The values were practically constant apart from a decrease observed at the early intervals after sham-irradiation, probably due to the handling of the animals.

Maltase and alkaline phosphatase were clearly modified after irradiation. Maltase activity at 6 to 10 h and alkaline phosphatase at 6 to 24 h were significantly higher than in the controls. At the remaining intervals, as with LAP activity, the curve referring to irradiated animals had the same course as the control curve, but the values were higher.

Only 74 h after irradiation maltase and alkaline phosphatase activities were lower.

Lysosomal enzymes and protein content fluctuations observed in controls killed at different intervals were neither significant nor sufficient to demonstrate a

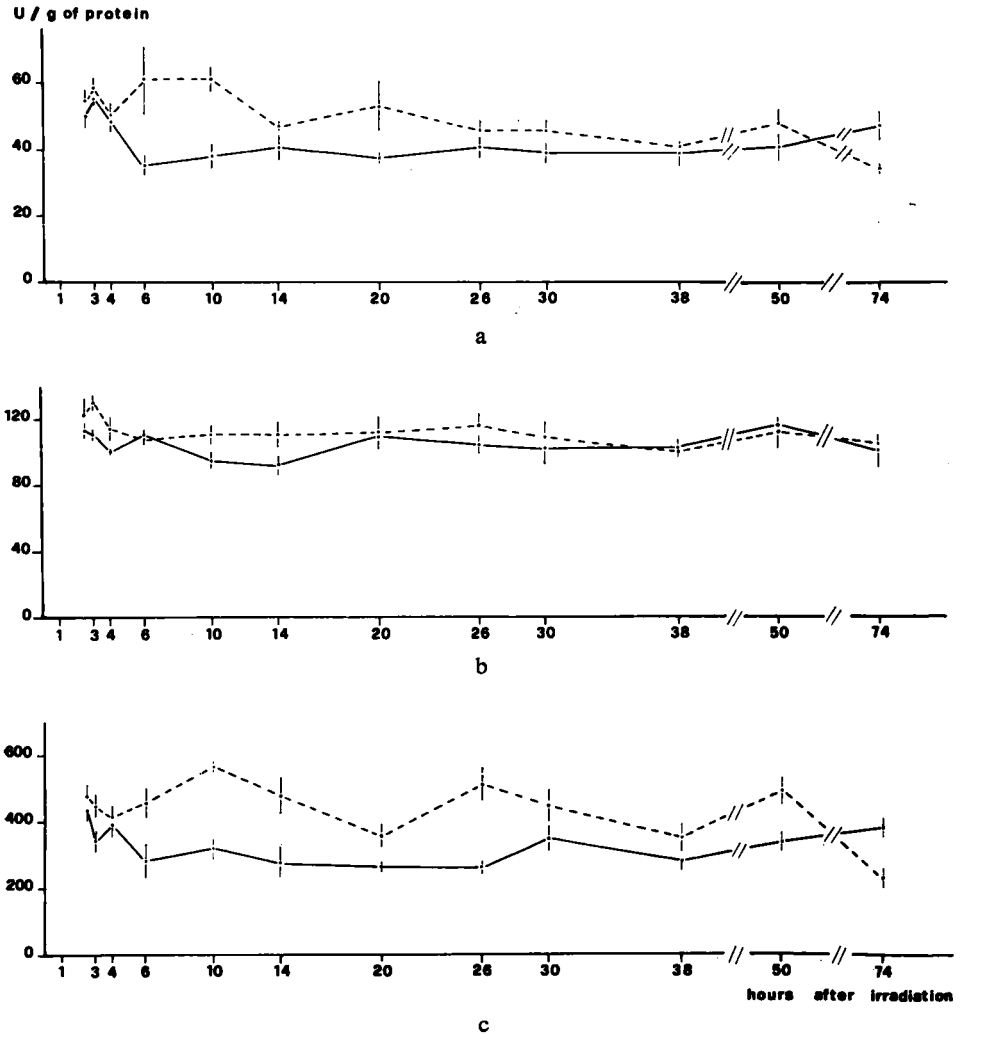

Fig. 1. First experiment. a) Maltase, b) LAP and c) alkaline phosphatase activities. Mean value ± SE. --- Irradiated. —— Control.

circadian rhythm of these activities (Fig. 2). Cathepsin D generally appeared reduced after irradiation although not in a significant way. β-glucuronidase instead was significantly increased only at 14 and 30 h after irradiation (Fig. 2). Protein content of the homogenate was practically constant during the entire experiment. The values from the irradiated animals overlap those from the controls with some fluctuations.

Morphologically a modest tubular enlargement was observed at the early intervals after irradiation and a slight inflammation at 4 h persisted throughout the rest of

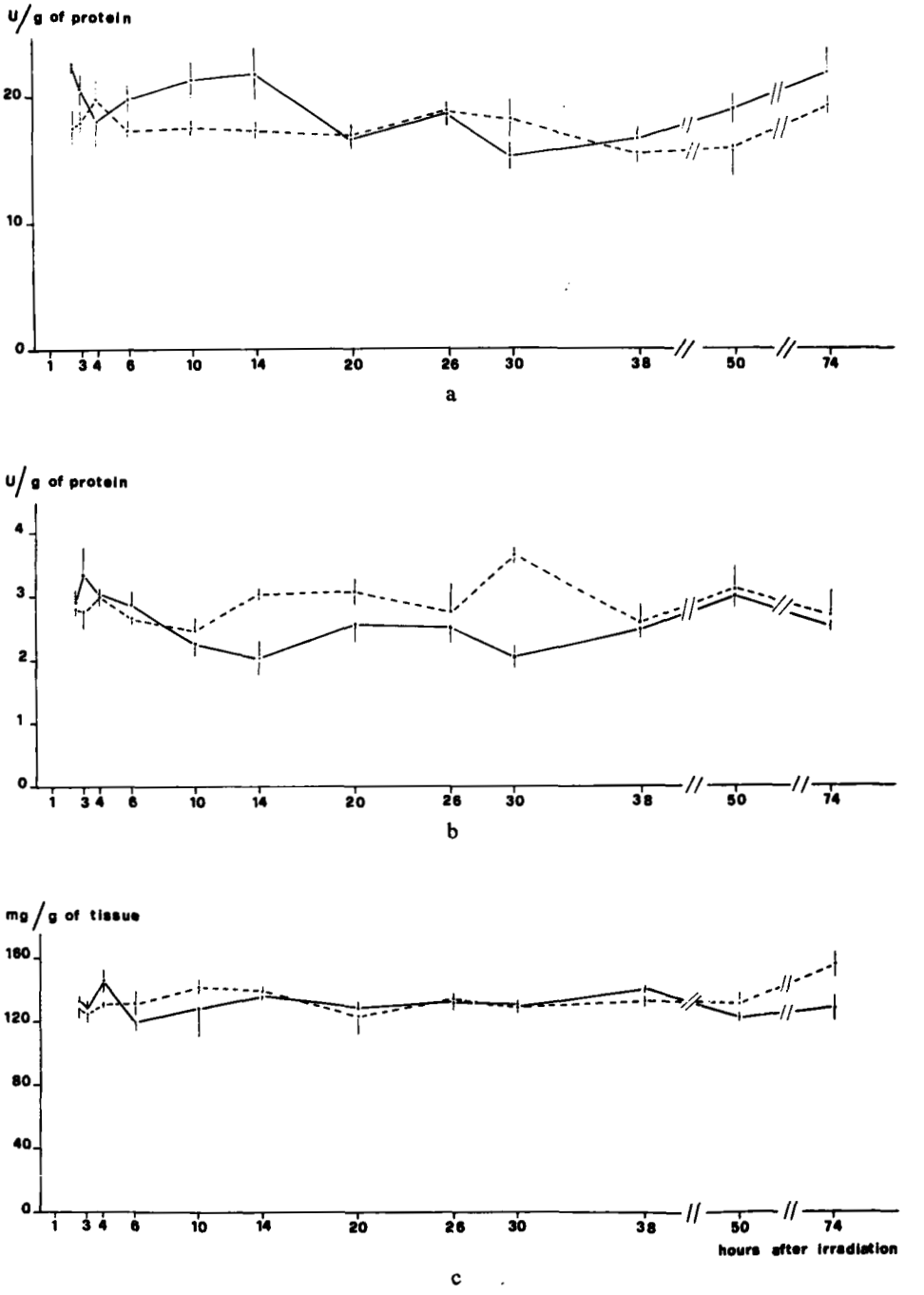

Fig. 2. First experiment. a) Cathepsin D, b) β-glucuronidase activities and c) protein content. Mean value $\pm$ SE. --- Irradiated . —— Controls.

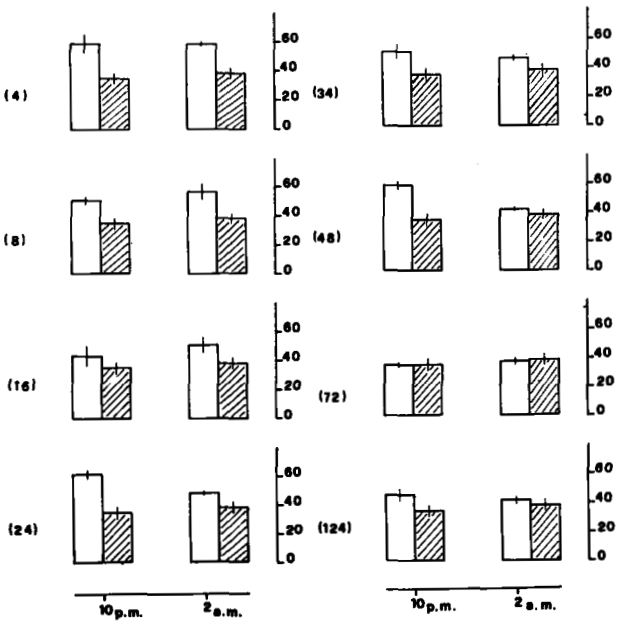

Fig. 3. Second experiment. Maltase activity (U/g of protein). Mean value ± SE. Time between irradiation and killing of the first group in parentheses. ☐ Irradiated. ▨ Controls.

the experiment. No evident nuclear or cytoplasmatic modifications of the cells of the renal parenchyma were found at histologic examination.

Second experiment. The object of this experiment was to analyse whether irradiation at different hours induces modifications different from those investigated previously.

Maltase activity (Fig. 3) increased significantly in the first two groups. When the time between irradiation and killing was higher, this increase was still present and appeared marked in some of the rats killed at 10 p.m.

LAP results overlap those of the first experiment. In the irradiated animals activities did not differ significantly from the controls.

Alkaline phosphatase (Fig. 4) had the same course as maltase activity: a significant increase occurred in the early groups. At subsequent intervals the values appeared similar to the controls except at 5 d after irradiation, when a decrease was found.

Also lysosomal enzyme results overlap the values from the first experiment. β-glucuronidase had values higher than the controls although not generally significant. Instead cathepsin activity appeared slightly reduced. This decrease was more evident at the later intervals (Fig. 5).

The values of protein content after irradiation appeared similar to those from the first experiment. In the animals irradiated 74 and 124 h before killing the protein content was significantly higher than in the controls.

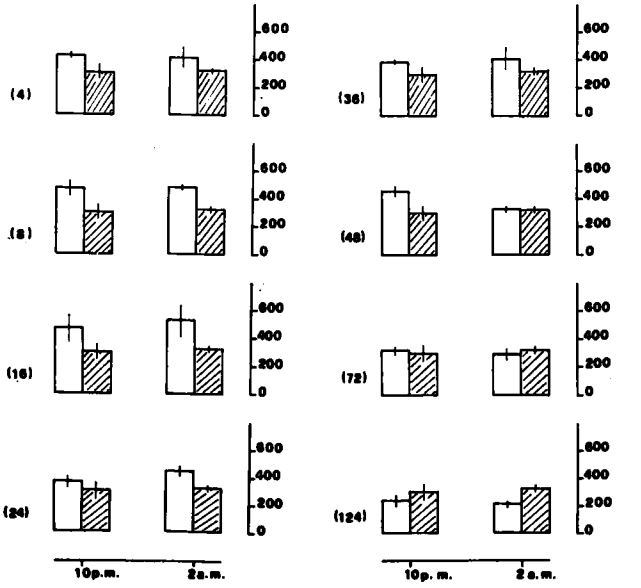

Fig. 4. Second experiment. Alkaline phosphatase activity (U/g of protein). Mean value ± SE. Time between irradiation and killing of the first group in parentheses. ☐ Irradiated. ▨ Control.

Discussion

Absorption of disaccharases and dipeptidases takes place in the proximal tubules of the kidney after hydrolysis in monomeric forms produced by specific enzymes localized in the brush border membranes; otherwise these molecules would be lost in the urine (SACKTOR, SILVERMANN, SILVERMANN & BLACK, THOMAS & KINNE 1972). This activity is similar to that observed in the differentiated cells of small intestinal epithelium and is called membrane digestion (UGOLEV & DE LAEY 1973).

Previous investigations (BECCIOLINI et coll. 1972, 1973, 1976 b, 1977) have indicated a significant increase in brush border enzyme activities a few hours after irradiation until morphologic alterations occurred at the top of the villi. Very low disaccharase and dipeptidase activities and high lysosomal activities appear when the villi are flattened and the epithelium heavily altered. The mucosa returns to normal morphology when enzyme activities reach the same levels as in the control animals.

The present results confirm the absence of circadian fluctuations in brush border and lysosomal enzyme activities in kidneys. Moreover, the experiments show that ionizing radiation affects some activities of the proximal tubule. Maltase, alkaline phosphatase and β-glucuronidase tend to increase, but only during some of the early intervals is this increase significant. LAP, cathepsin D and protein content are not evidently modified. However it is worth noting that alkaline phosphatase and LAP are localized not only in the brush border of proximal tubules but also in other renal structures (QUIRK 1972).

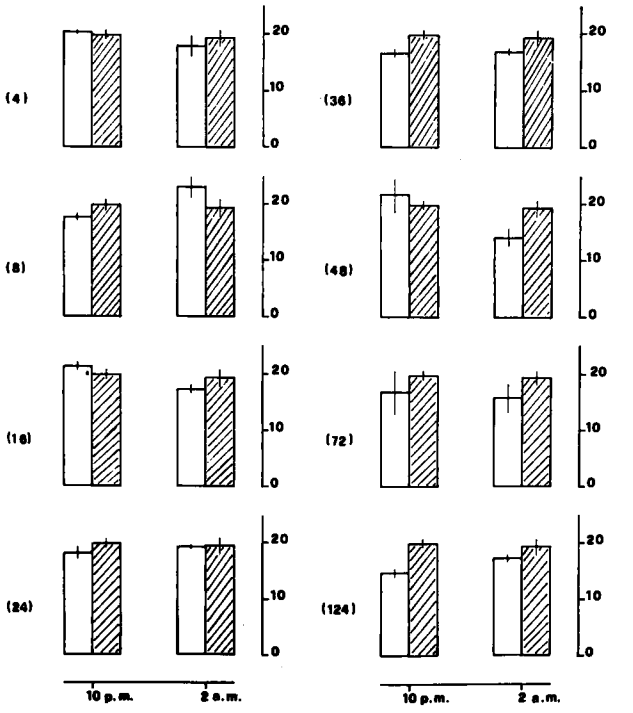

Fig. 5. Second experiment. Cathepsin D activity (U/g of protein). Mean value ± SE. Time between irradiation and killing of the first group in parentheses. ☐ Irradiated. ▨ Control.

The absence of evident modifications in the lysosomal enzymes (taken as cell damage index) and in protein content is a further evidence of normal morphology present throughout the experiments. The difference in behaviour between enzymes with similar activities and localized in structures having similar functions but belonging to epithelia with different proliferative activity and turnover was confirmed. This difference may explain the weak response of the kidney as compared to that of the small intestine irradiated with the same dose.

The results appear to agree with a recent suggestion (BECCIOLINI et coll. 1976 b) on the modifications of intestine enzymes. The increase of intestinal enzyme activities seems to be due neither to the cells differentiated at the moment of irradiation, nor to the proliferative ones which are still lacking enzymes. Only cells irradiated at a certain stage of the differentiation process would be responsible for the increase in enzyme activities of the brush border. These cells are very few in the renal parenchyma, and the increase phase does not appear as evident and lasting as in the small intestine.

SUMMARY

Brush border enzymes of proximal tubules, lysosomal activities and protein content of rat kidney were analysed after whole-body irradiation using two different experimental

schedules. Maltase, alkaline phosphatase and β-glucuronidase activities increased moderately during the first days after irradiation, whereas LAP, cathepsin D activities and protein content were not modified. No evident morphologic alterations were observed.

ZUSAMMENFASSUNG

Die Kantenenzyme der proximalen Tubuli, die lysosomalen Aktivitäten und der Proteingehalt der Rattenniere nach Gesamtkörperbestrahlung wurde unter Verwendung von zwei unterschiedlichen experimentellen Schemata analysiert. Die Maltase, alkalische Phosphatase- und β-Glucuronidase-Aktivitäten stiegen mässig während der ersten Tage nach der Bestrahlung, während LAP, Cathepsin D Aktivitäten und der Proteingehalt sich nicht änderten. Keine klaren morphologischen Veränderungen wurden beobachtet.

RÉSUMÉ

Les auteurs ont étudié après irradiation totale du corps au moyen de 2 schémas expérimentaux différents les enzymes des bordures en brosse des tubules proximaux, les activités lysosomales et le contenu en protéine du rein du rat. Les activités de la maltase, de la phosphatase alcaline et de la β-glycuronidase augmentent modérément au cours des premiers jours après l'irradiation alors que les activités LAP, cathepsine D et le contenu en protéine ne sont pas modifiés. Les auteurs n'ont pas observé d'altération morphologique évidente.

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